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Coplanar PCB Congeners Increase Uterine Weight and Frontal Cortical Dopamine in the Developing Rat: Implications for Developmental Neurotoxicity

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We show that developmental exposure of the laboratory rat to the coplanar polychlorinated biphenyl (PCB) congener 3,4,3′,4′-tetrachlorobiphenyl (TCB) and the structurally similar congener 3,4,5,3′,4′-pentachlorobiphenyl (PtCB) elevates dopamine (DA) concentrations in the prefrontal cortex (PFC). To determine whether these coplanar congeners are estrogenic, and may thus contribute to the elevations in PFC DA, we measured uterine wet weight (UWW) in prepubertal rats exposed to TCB or PtCB. For comparison, additional animals were exposed to either the *ortho*-substituted congener 2,4,2′,4′-tetrachlorobiphenyl (*o*-TCB) or 3,4,5,3′,4′,5′-hexachlorobiphenyl (HCB), a coplanar congener highly resistant to metabolism. Both TCB and PtCB increased UWW, but this effect was blocked after exposure to the anti-estrogen ICI 182,780. Neither *o*-TCB nor HCB altered UWW. These results demonstrate that certain coplanar PCB congeners and/or their metabolites, are estrogenic, and suggest that exposure during critical periods of neuronal development may increase central DA concentrations, and by inference, alter behavior.

Key Words: **polychlorinated biphenyls; coplanar; uterotropic; brain; dopamine; developmental neurotoxicity.**

INTRODUCTION

Developmental exposure to polychlorinated biphenyls (PCBs), a class of widely dispersed and persistent environmental contaminants (Erickson, 1997), is associated with altered development in children (Darvill *et al.*, 2000; Huisman *et al.*, 1995; Jacobson and Jacobson, 1996; Winneke *et al.*, 1998) and with behavioral and neurochemical changes in experimental animals (Kaya *et al.*, 2002; Roegge *et al.*, 2000; Seegal, 2003). These behavioral and neurochemical responses to PCBs are, to a large extent, dependent on the structure of the congener, *i.e.*, whether the congener is coplanar or non-coplanar (Hany *et al.*, 1999; Seegal *et al.*, 1997).

For example, non-coplanar congeners alter neurotransmitter function (Bemis and Seegal, 2004; Shain *et al.*, 1991) and regulation of intracellular signaling systems, including calcium, in both experimental animals and tissue culture (Bemis and Seegal, 2000; Seegal, 2003). Coplanar congeners, on the other hand, are largely devoid of activity when exposure occurs either in the adult animal or in the majority of *in vitro* systems (Kodavanti *et al.*, 1995; Mariussen *et al.*, 1999; Shain *et al.*, 1991; Wong *et al.*, 1997), but they are neurochemically active after developmental exposure of laboratory rodents (Agrawal *et al.*, 1981; Seegal *et al.*, 1997). Thus, coplanar PCB congeners are true neuroteratogens, *i.e.*, agents that alter function when administered during development, but that are devoid of activity when presented to the adult.

One possible explanation for the neuroteratogenic actions of this class of congeners is their ability to act as endocrine disrupters (Amin *et al.*, 2000). For example, developmental exposure of laboratory rats to coplanar PCBs reduces circulating concentrations of triiodothyrodine (T3) and thyroxine (T4) (Goldey *et al.*, 1995; Morse *et al.*, 1993), whereas epidemiological studies demonstrate a relationship between developmental exposure to PCBs and decreased circulating levels of thyroid hormones (Koopman-Esseboom *et al.*, 1994). However, reductions in circulating levels of thyroid hormones are associated with decreases in central dopamine (DA) concentrations (Puymirat, 1985) and thus are not likely to contribute to the elevations in PFC DA that we have observed.

A more likely mechanism by which PCB congeners could increase PFC DA concentrations is by acting as an estrogen agonist (Garner *et al.*, 1999; Korach *et al.*, 1988; Layton *et al.*, 2002). Indeed, there is an extensive literature demonstrating that estradiol increases DA turnover (Di Paolo *et al.*, 1985) as well as DA concentrations in the basal ganglia of ovariectomized rats exposed to estradiol (Pasqualini *et al.*, 1995). Although these data have been obtained from adult animals, the results that we present here demonstrate that developmental exposure to either TCB (3,4,3′,4′-tetrachlorobiphenyl) or PtCB (3,4,5,3′,4′-pentachlorobiphenyl) also results in prolonged increases in PFC DA. We thus undertook a series of

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studies to determine whether coplanar PCB congeners that elevate PFC DA after developmental exposure do so because of their ability to act as estrogen agonists.

MATERIALS AND METHODS

Subjects. For the developmental neurochemical studies, precisely timed pregnant Sprague-Dawley rats from Zivic-Miller Laboratories, Inc. (Zelienople, PA), were exposed to varying concentrations of TCB, PtCB, or *ortho*-TCB from gestational day 6 through weaning. There were a minimum of 7 litters per experimental treatment.

For the prepubertal UWW experiments, subjects consisted of female Sprague-Dawley derived rats from the breeding facilities of the New York State Department of Health. Litters were received on postnatal day (PND) 18 and weaned on PND 21, at which time they were injected intraperitoneally (ip) with either corn oil (vehicle) or corn oil containing various concentrations of TCB, PtCB, HCB (3,4,5,3′,4′,5′-hexachlorobiphenyl), or *ortho*-TCB (2,4,2′,4′-tetrachlorobiphenyl). In additional experiments, animals were exposed to the congener of interest and injected ip with 0.5 mg/kg of the estrogen receptor antagonist (ICI 182,780) in corn oil on PND 21–23.

Developmental exposure to PCBs. All congeners were purchased from AccuStandard (New Haven, CT) and were at least 99.8% pure. Dams were exposed to PCBs on a daily basis by placement of measured amounts of corn oil containing TCB, PtCB, or *o*-TCB onto a half of a vanilla wafer cookie. The volume of PCB-corn oil solution added to the cookies (100 μl/200 g body weight) was adjusted twice weekly based on the body weights of the dams. This technique has been used in our laboratory for a number of years and has been found to be a reliable, non-stressful means of exposing rats to the contaminants (Seegal *et al.*, 1997). The concentrations of PCBs used in these studies were based on dose-ranging studies that determined the acute toxicities of the congeners. The congeners were administered as follows: TCB at doses of 0.1 or 1 mg/kg/day; PtCB at doses of either 0.25 or 1 μg/kg/day; and *o*-TCB at doses of 1, 10, or 20 mg/kg/day. Control dams received cookies containing only corn oil.

Developmental neurochemical studies. Litters were culled to four male and four female pups within 24 h of birth and were supplemented at that time, if necessary, with additional offspring from litters receiving the same treatment. One male and one female from each litter were sacrificed on PND 35, 60, and 90 by stunning and decapitation. Procedures were approved by the Wadsworth Center Institutional Animal Care and Use Committee. The brains were rapidly removed, rinsed with ice-cold saline, blotted, and frozen on dry ice and maintained at −80°C until dissection and neurochemical analysis.

Frozen brains were brought to −10°C and tissue samples from the prefrontal cortex (PFC) were obtained by free-hand dissection according to the brain dissection guide of Palkovits and Brownstein (1988). Samples were weighed and homogenized in 20 volumes (w/v) of ice-cold 0.2 N perchloric acid containing 100 mg/l EGTA. Dopamine concentrations were determined by high-performance liquid chromatography with electrochemical detection according to procedures described by our laboratory (Bemis and Seegal, 1999). Detection limits (defined as twice baseline) were 20–25 pg/injection. Data were electronically recorded using Waters Millennium Chromatography Manager software (Milford, MA), and the chromatographic peaks were integrated and reviewed prior to statistical analysis.

PCB exposure for prepubertal UWW experiments. TCB, PtCB, HCB, and *o*-TCB were dissolved in corn oil and administered ip at a volume of 200 μl/kg on PND 21 and 22. TCB was administered ip at concentrations resulting in exposure to 3, 9, or 27 mg/kg/day; PtCB was administered ip at concentrations resulting in exposure to 4, 16, 64, 100, 200, 300, or 400 μg/kg/day; HCB was administered ip at concentrations resulting in exposure to 200, 400, or 800 μg/kg/day; and *o*-TCB was administered ip at concentrations resulting in exposure to 8, 16, or 32 mg/kg/day. These doses were selected on the basis of doses reported either in previous studies (Nesaretnam *et al.*, 1996) or in pilot studies that we conducted to determine the range of doses that altered UWW in the absence of overt signs of toxicity. Exposures were distributed across animals within a litter, such that all conditions were represented (*i.e.*, each female in a litter was exposed to a different concentration of the same PCB congener). In all experiments, control animals received corn oil injections according to the same protocol. On PND 24 body weights were determined and the animals sacrificed using procedures approved by the Wadsworth Center Institutional Animal Care and Use Committee. Uteri were rapidly removed, dissected free of fat and connective tissue, and weighed. The dissections were blinded, in that the investigator carrying them out was aware of neither the body weight of the animal nor the treatment that it had received.

Statistical procedures: developmental neurochemical studies. Data were analyzed using three-way analysis of variance (ANOVA) procedures that allowed determination of the effects of dose, age, and gender, as well as interactions between these main effects. *Post hoc* Bonferroni-corrected *t*-tests were carried out to determine which groups differed significantly from others.

Statistical procedures: postnatal UWW experiments. Uterine wet weight data from replicate experiments for each congener were combined for statistical analyses. The experimental results were expressed as either UWW or the ratio of UWW to body weight (BW), because these congeners, particularly at the higher doses, reduced body weight and could thus complicate interpretation of results if the data were not corrected for body weight. Data were statistically analyzed using both one-way and two-way ANOVA, combined with *post hoc* Bonferroni-corrected *t*-tests.

RESULTS

Neurochemical Effects of Developmental Exposure to PCBs

For the congeners tested, there were no significant interactions between the gender of the offspring and PCB exposure. Thus, for purposes of clarity, we analyzed the results of developmental exposure to the coplanar and non-coplanar congeners by combining data from both males and females, while maintaining age as a dependent variable. *In utero* and lactational exposure to either TCB or PtCB significantly increased DA concentrations in the PFC; for TCB ($F = 7.21$; $df = 2,19$; $p \leq 0.01$) and for PtCB ($F = 3.90$; $df = 2,135$; $p \leq 0.05$) (Table 1).

In contrast to the elevations in PFC DA seen with the above coplanar congeners, developmental exposure to *o*-TCB significantly decreased DA concentrations in the PFC ($F = 4.86$; $df = 3,41$; $p \leq 0.01$), although the reductions in PFC DA persisted only through PND 60 (see also Table 1).

Effects of Prepubertal Exposure to PCBs on Uterine Weight

TCB dose-dependently increased UWW ($F = 7.65$; $df = 3,40$; $p \leq 0.001$) and the ratio of UWW/BW ($F = 9.11$; $df = 3,40$; $p \leq 0.001$) while significantly decreasing BW ($F = 4.37$; $df = 3,40$; $p \leq 0.01$; Fig. 1A–1C). *Post hoc* analyses of the data indicated that the highest exposure differed significantly from the corn oil controls for all three measures ($p \leq 0.01$). At the highest dose, BW was decreased by approximately 9%, relative to controls.

TABLE 1
Frontal Cortical Dopamine Concentrations (ng/mg tissue) in Offspring Developmentally Exposed to Three Polychlorinated Biphenyl Congeners

Congener	Dose	Age: 35 Days	Age: 60 Days	Age: 90 Days
3,4,3′,4′-TCB	Control	0.039 ± 0.002	0.052 ± 0.004	0.067 ± 0.005
	0.1 mg/kg	0.045 ± 0.002	0.063 ± 0.003	0.082 ± 0.003*
	1 mg/kg	0.048 ± 0.002*	0.073 ± 0.007**	0.090 ± 0.005***
3,4,5,3′,4′-PtCB	Control	0.044 ± 0.002	0.070 ± 0.005	0.064 ± 0.004
	0.25 μg/kg	0.045 ± 0.002	0.069 ± 0.003	0.063 ± 0.003
	1 μg/kg	0.054 ± 0.003**	0.066 ± 0.002	0.077 ± 0.003***
2,4,2′,4′-TCB	Control	0.037 ± 0.002	0.055 ± 0.002	0.066 ± 0.002
	1 mg/kg	0.033 ± 0.002	0.049 ± 0.003	0.061 ± 0.002
	10 mg/kg	0.035 ± 0.002	0.049 ± 0.004	0.067 ± 0.004
	20 mg/kg	0.026 ± 0.001***	0.041 ± 0.002**	0.061 ± 0.004

*$p \leq 0.05$,
**$p \leq 0.01$,
***$p \leq 0.001$; Bonferroni-corrected *t*-tests comparing each PCB-treated group with its age-matched control. Data for 3,4,3′,4′-TCB and 2,4,2′,4′-TCB adapted from Seegal *et al.* 1997.

Exposure to PtCB also dose-dependently increased UWW ($F = 8.55$; $df = 7{,}121$; $p \leq 0.001$) and the ratio of UWW/BW ($F = 18.28$; $df = 7{,}121$; $p \leq 0.001$) while decreasing BW ($F = 8.25$; $df = 7{,}121$; $p \leq 0.001$; Fig. 2A–2C). *Post hoc* analyses indicated that all concentrations equal to or greater than 100 μg/kg significantly increased UWW and UWW/BW and significantly decreased BW relative to control animals. The decrease in BW at the highest dose was approximately 15%, relative to control animals.

Exposure to either the coplanar congener HCB or the non-coplanar congener *o*-TCB, on PND 21 and 22, had no discernible effects on either UWW or UWW/BW (data not shown).

ICI 182,780 Blocks Increases in UWW after Exposure to TCB and PtCB

ICI 182,780 reduced UWW by approximately 20% in control animals, and when administered ip on PND 21–23, completely blocked the approximately 50% increase in UWW in animals exposed to the highest doses of either TCB or PtCB (Fig. 3).

DISCUSSION

These studies demonstrate that developmental exposure to TCB or PtCB significantly increases PFC DA, whereas the non-coplanar congener *o*-TCB reduced PFC DA concentrations. We hypothesize that these elevations are due to the estrogenic actions of either the parent coplanar congener or its hydroxylated metabolites. To test that hypothesis, we determined whether these congeners increased UWW in the prepubertal rat, a measure that is considered the "gold standard" in testing for estrogenicity (Bachman *et al.*, 1998).

We now report that prepubertal exposure to TCB and PtCB significantly elevates UWW. The lack of effect of *o*-TCB on UWW, combined with the reductions in PFC DA after *in utero* and lactational exposure to this non-coplanar PCB congener, further supports the above hypothesis.

We also determined whether prepubertal exposure to TCB and PtCB elevated PFC DA. These congeners, administered postnatally, had no measurable effects on brain DA concentrations (data not shown). These results are not surprising because we (Seegal, 2001) have previously shown that central DA function in the adult is insensitive to these congeners. Thus, the elevations in PFC DA after developmental exposure to TCB or PtCB most likely reflect the greater sensitivity of the developing central nervous system (CNS) to environmental estrogens, relative to either the mature or early post-weaning brain. Indeed, it is our contention that "estrogenic" PCB congeners yield long-term changes in brain DA independent of the brain concentrations of PCBs at the time of sacrifice by altering the organization of the brain. Thus, estrogenic PCBs are hypothesized to act in a manner similar to steroid hormones administered during development that have an organizational role leading to permanent changes in brain function.

Despite the findings on UWW reported here, there is still a lack of consensus concerning whether coplanar congeners are uterotropic. Indeed, our results, demonstrating that prepubertal exposure to TCB and PtCB significantly increases UWW and UWW/BW, add to the disparate literature concerning the possible estrogenic activity of coplanar PCB congeners. Both Nesaretnam and colleagues (1996) and we have shown that TCB significantly elevates UWW (although in different species),

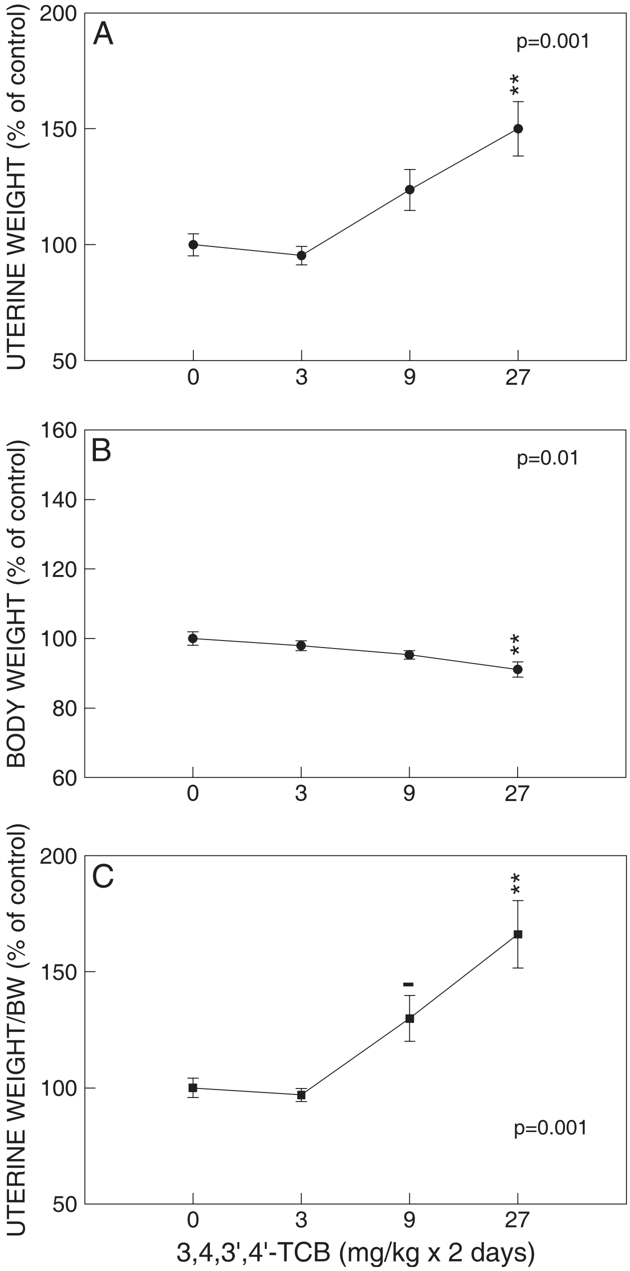

FIG. 1. Effect of 3,4,3′,4′-TCB on (A) uterine weight, (B) body weight, and (C) uterine weight/body weight ratio determined on postnatal day (PND) 24. All data are expressed as a percent of uterine weight in corn oil-treated controls. Animals were injected with 9–27 mg/kg of 3,4,3′,4′-TCB or corn oil (control) on PND 21 and PND 22 as described in *Methods*. N = 8–16 animals per treatment; $^{**}p \leq 0.01$, $-p \leq 0.1$, significantly different from control using *post hoc* Bonferroni-corrected *t*-tests.

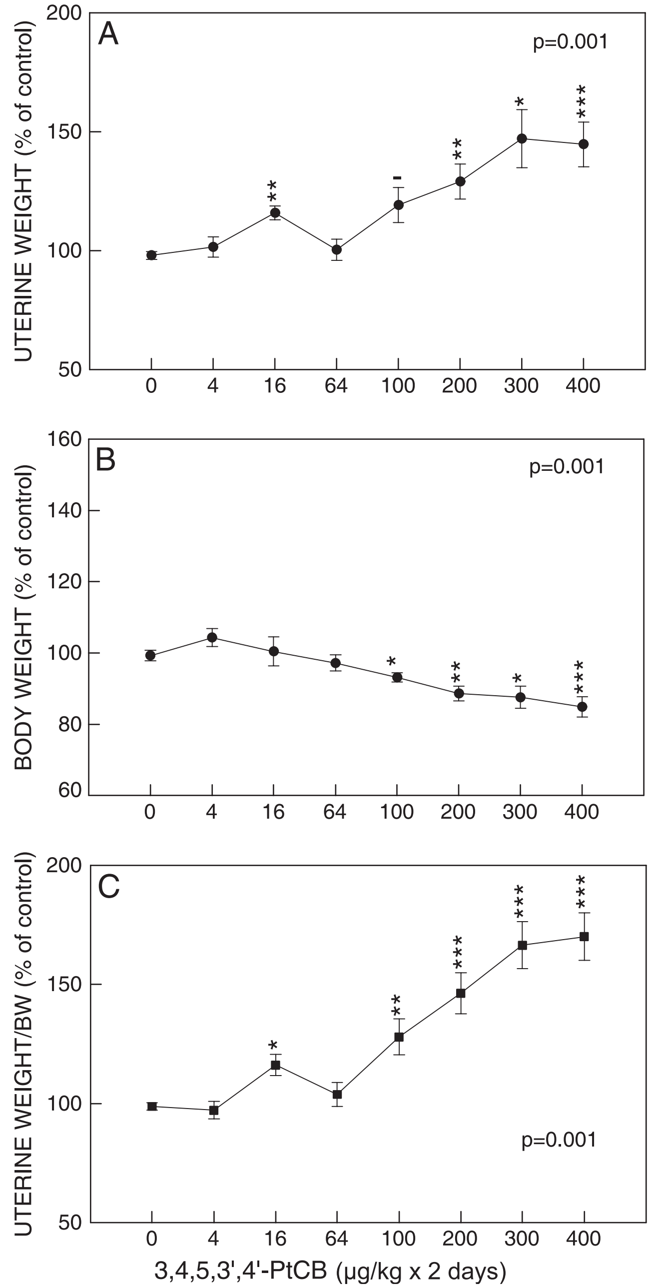

FIG. 2. Effect of 3,4,5,3′,4′-PtCB on (A) uterine weight, (B) body weight, and (C) uterine weight/body weight ratio determined on PND 24. All data are expressed as a percent of uterine weight in corn oil-treated controls. Animals were injected with 4–400 µg/kg of 3,4,5,3′,4′-PtCB or corn oil (control) on PND 21 and PND 22 as described in *Methods*. N = 9–31 animals per treatment; $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$, $-p \leq 0.1$, significantly different from control using *post hoc* Bonferroni-corrected *t*-tests.

whereas Jansen and co-workers (1993) and Ramamoorthy and co-workers (1999) failed to demonstrate that TCB was uterotropic in the prepubertal rat model. One factor that may contribute to the lack of agreement between laboratories is the dose of the congener employed in the various studies. We exposed prepubertal rats ip to TCB at concentrations ranging from 9 to 27 mg/kg/day for 2 days, whereas the studies demonstrating that TCB was inactive, either exposed rats to TCB at doses of 160 µg/kg for 2 days (PND 20–21) (Jansen *et al.*, 1993) or else exposed B6C3F1 mice to total doses ranging from 50 to 150 mg/kg over 3 days (PND 21–23) (Ramamoorthy *et al.*, 1999). Thus, if for the moment, we

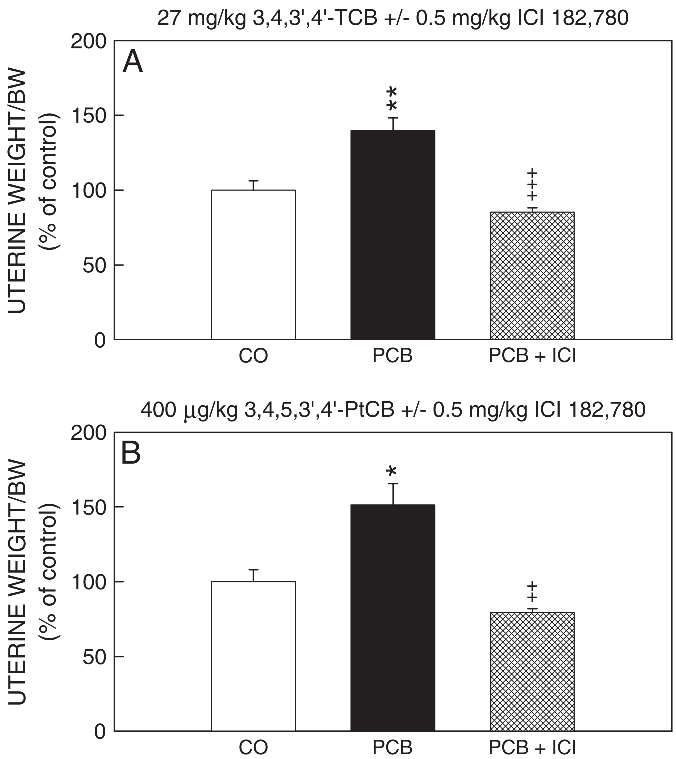

FIG. 3. Effect of ICI 182,780 on uterine weight/body weight ratios determined on PND 24 in animals exposed to 3,4,3′,4′-TCB or 3,4,5,3′,4′-PtCB. All data are expressed as a percent of uterine weight in corn oil-treated controls. (A) Animals were injected with 27 mg/kg of 3,4,3′,4′-TCB or corn oil on PND 21 and PND 22. Half of the animals that received 3,4,3′,4′-TCB also received injections of 0.5 mg/kg ICI 182,780 on PND 21 and PND 22. On PND 23 animals that received ICI 182,780 were given a third injection; all other animals received a corn oil injection. (B) Animals were injected according the design described above for panel A with 400 µg/kg of 3,4,5,3′,4′-PtCB. $N = 2–8$ animals per treatment; $^{**}p \leq 0.01$, $^{*}p \leq 0.05$, significantly different from control; $+++p \leq 0.001$, $++p \leq 0.01$, significantly different from exposure to the respective PCB congener.

ignore differences that might be attributable to either the species or the strain of animal used, the above data suggest that TCB is uterotropic over a fairly narrow range of doses. The data from Nesaretnam and co-workers (1996) are, however, more difficult to interpret, because those workers exposed mice to TCB by dissolving the congener in ethanol and applying this mixture to the animals' skin. In those studies, total doses ranged from 438 ng to 438 µg/day for 3 days, which on a per-kg basis (assuming that mice weigh approximately 30 g), resulted in exposures ranging from 14.6 µg/kg/day to 14.6 mg/kg/day. Nevertheless, despite the relatively low exposure to TCB, those authors noted significant elevations in UWW, suggesting that the route of administration may also influence the estrogenicity of TCB, perhaps by altering the rate of metabolism of the congener.

The age of the animal may also influence whether TCB is uterotropic. Nesaretnam and colleagues (1996) demonstrated that TCB was uterotropic when administered on PND 25–27, but was without effect when administered on PND 21–23. These authors suggested that, because of the relative immaturity of the PND 21 mouse liver, the younger animal was less able to metabolize the parent congener to an estrogenically more active form. However, age-dependent changes in the sensitivity of the uterus to coplanar PCBs cannot be ruled out.

PtCB was also estrogenic, and it significantly elevated UWW at doses above 16 µg/kg/day. These findings differ from those of Lind and co-workers (1999), who failed to demonstrate alterations in UWW in adult ovariectomized rats exposed for 3 months to PtCB (4 µg/kg/day), resulting in a total dose of 384 µg/kg. These discrepancies may reflect both the age of the animal at the time of exposure (the majority of studies have used the prepubertal rat) and the level of exposure of the animal to the congener.

ICI 182,780 Blocks Increases in UWW

ICI 182,780, a recognized estrogen receptor antagonist (Dukes *et al.*, 1992), blocked the increases in UWW and UWW/BW after exposure to either TCB or PtCB. These results suggest that, for these congeners to increase UWW, they (or their metabolites) must bind to the estrogen receptor (Korach *et al.*, 1988). This latter point (*i.e.*, the involvement of PCB metabolites) is supported both by Morse and colleagues (1995), who have demonstrated that perinatal exposure of the laboratory rodent to TCB resulted in significant formation and retention of 3,5,3′,4′-tetrachloro-4-biphenylol, and by Koga and colleagues (1990), who have shown that PtCB can also be metabolized to hydroxylated metabolites. In turn, Korach and co-workers (1988) have demonstrated that hydroxylated metabolites of PCBs bind with varying degrees of avidity to the estrogen receptor. Additional, albeit indirect evidence, that hydroxylated metabolites, rather than the parent congeners, are estrogenic is provided by our findings that HCB, a coplanar congener highly resistant to metabolism (Kohli *et al.*, 1979), was without effect when administered to prepubertal rats at concentrations comparable to or higher than concentrations employed for PtCB. However, an alternative to the hypothesis that PCB congeners or their metabolites must bind to the estrogen receptor has been suggested by Kester and co-workers (2000). These investigators demonstrated *in vitro* that (1) hydroxylated PCB metabolites potently inhibit estrogen sulfotransferase, thereby increasing estrogen bioavailability and (2) this inhibition occurs at much lower levels than does binding of the hydroxylated PCB metabolite to the estrogen receptor.

Lack of Effect of o-TCB on UWW

In contrast to the elevations in PFC DA and UWW seen after prepubertal exposure to TCB or PtCB, *o*-TCB did not alter either UWW or UWW/BW, clearly demonstrating that this non-coplanar PCB congener is not estrogenic. However, this lack of any discernible effect on UWW illustrates the difficulty in predicting the estrogenicity of PCBs, because Jansen and coworkers (1993) demonstrated that a structurally similar non-coplanar congener (2,5,2′,5′-tetrachlorobiphenyl) increased UWW in prepubertal rats.

In summary, we have shown that the coplanar congeners TCB and PtCB elevate PFC DA after developmental exposure and increase UWW in the prepubertal rat model. These data, combined with the lack of effect of HCB, a congener highly resistant to metabolism, on UWW and the reductions in PFC DA seen with the non-coplanar congener *o*-TCB, support our hypothesis that only coplanar PCB congeners exert their influences on neurochemical function by acting as estrogen agonists. There are, at the very least, two possible mechanisms by which estradiol and environmental estrogens could increase central dopamine concentrations. First, as alluded to previously, increases in circulating estrogens have been shown to increase DA concentrations by increasing DA turnover, thus increasing activity of the rate-limiting enzyme, tyrosine hydroxylase. Second, the increases in DA concentrations seen here after developmental exposure to estrogenic PCB congeners may also reflect a reduction in the developmentally programmed loss of DA neurons that occurs during normal brain development as a result of the well-known neuroprotective role of estradiol.

In conclusion, these data add to the body of literature suggesting that certain environmental contaminants may influence development and CNS function by mimicking the actions of estradiol, albeit at concentrations much higher than the natural ligand.

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